Comments to the Editor

Transient Complexes between Dark Rhodopsin and Transducin: Circumstantial Evidence or Physiological Necessity?

In a recent article that appeared in the *Biophysical Journal*, Schöneberg et al. (1) built up a physical model describing in spatiotemporal detail the early molecular steps in phototransduction. These steps include absorption of photons by the receptor rhodopsin (R) and the subsequent activation of a G protein-mediated signaling cascade in photoreceptor cells. The interaction of R and the G protein transducin (G) is prototypical for G protein-coupled receptor (GPCR) systems and the generation of accurate computational models of phototransduction is of high importance for the general understanding of GPCR function. Schöneberg et al. (1) used particle-based reaction diffusion simulations to build their model. Based on previously performed simulations by Dell'Orco and Schmidt (2), the analysis by Schöneberg et al. (1) was significantly improved by introducing reactivity between molecules, thus allowing a better assessment of the implications for the visual cascade kinetics. A challenging aspect of this approach was to simulate the effects of supramolecular arrangements of R on the kinetics of the photoresponse. The main conclusions in that article are as follows.

1. The classical model, in which R and G are free to diffuse in the membrane milieu and trigger the cascade by a collisional coupling mechanism, is consistent with available kinetic data on the activation of G by photoactivated R (R*).
2. The supramolecular architecture of R in racks of dimers has modest impact on the kinetics of the cascade, because diffusivity of G can apparently override the influence of the receptor architecture in the case of immobile R molecules.
3. The existence of transient complexes formed by R and G before light stimulus hypothesized and demonstrated in earlier studies (see Dell'Orco (3) for a recent Review) is only consistent with the phototransduction kinetics if the precomplexes are weak and dissociate with a relatively large rate.

While we appreciate the important purpose and the methodology of the work, we do not generally agree with the conclusions in that article, because we believe that some of the recent data concerning the supramolecular organization of R and R/G in disks have not been properly implemented in the model. In particular, we argue that the way the dark RG transient complexes have been considered in that work does not reflect the actual set of available experimental data. Therefore, we shall now clarify our concerns and discuss the physiological role preformed complexes may have in vivo as a consequence of the supramolecular organization of R in photoreceptors.

On the implementation of RG preformed complexes in the dark

The lifetime of a putative preformed RG complex would be of critical importance for a fast and efficient signaling process like phototransduction. Schöneberg et al. (1) implemented the reaction kinetics of complex formation by setting the association rate constant k_{pre}^{micro} to the diffusion limit and by taking an experimentally reported dissociation rate constant k_{-pre} of 0.148 s^{-1}, which is a value determined in our previous work based on surface plasmon resonance (SPR) spectroscopy (5). The authors then made the quite straightforward observation that such a value would correspond to a lifetime for the RG complexes of 6.8 s, clearly far too long for allowing the physiological rate of G protein activation.

Based on this reasoning and on ordinary differential equation fitting to their experimental data (4), they then estimated k_{-pre} to be necessarily at least 11,200 s^{-1} (see Fig. S5 in the Supporting Material in Schöneberg et al. (1)), corresponding to 20% free G and 80% RG precomplexes. This value would be consistent with measured and well-documented kinetics of phototransduction. However, it should be noted that the experimental SPR setup used in our previous experiments (5) has well-known limitations in resolving fast dissociation processes. As documented in our publication (5), we were aware of that limit and emphasized that a value of k_{-pre} should be considered only in relative rather than absolute terms. We were also aware of the unique advantage offered by the on-chip approach to measure rates in a comparative fashion, on the very same sample and under different

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*Correspondence: daniele.dellorco@univr.it or karl.w.koch@uni-oldenburg.de

Daniele Dell'Orco's current address is Hanse-Wissenschaftskolleg (HWK) Institute for Advanced Study, Delmenhorst, Germany.

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conditions including experiments performed in complete darkness.

Direct measurements of the rate constants k^{on} and k^{off} in our SPR binding experiments (see Table 1 in Dell'Orco and Koch (5)), although very likely affected by the in vitro conditions, allowed us to establish simple but robust kinetic constraints when comparing the binding of the G protein to dark versus photoactivated R. We could in fact establish that the association between R and G occurs ~1.6-fold faster in the dark ($k^{on}{}_{dark} = 1.6 \times k^{on}{}_{light}$) and that the dissociation of RG complexes occurs at least 315-fold faster in the dark ($k^{off}{}_{dark} = 315 \times k^{off}{}_{light}$). The latter value should be considered a lower bound, due to the known limitations discussed above. To check these relative values for consistency with the kinetics of the overall phototransduction cascade, we integrated them in the most comprehensive mathematical model of phototransduction.

Specifically, our numerical simulations were implemented by using the same absolute values taken from a previous work (6) for the kinetic rates of binding of G to unphosphorylated photoactivated R* (corresponding to the parameter k_{G1_0} in Dell'Orco et al. (6)) and for the dissociation of R*G complexes (corresponding to the parameter k_{G2} in Dell'Orco et al. (6)) and by imposing on these values the kinetic constraints elucidated above. This brought us to determine the absolute values for the dark binding and dissociation of RG complexes that guaranteed the correct kinetics of the whole cascade.

Schöneberg et al. (1) used in their modeling approach for the rate of R*G complex dissociation a value of 200 s^{-1} (denoted k_{-1} or equivalently $k^{off}{}_{light}$), a value that is consistent with experimental parameters. What is then a reasonable value for the dissociation rate of a preformed RG complex in the dark? According to our line of arguments above, this value should be $k^{off}{}_{dark} = 315 \times k^{off}{}_{light}$, resulting in $315 \times 200\ s^{-1} = 63{,}000\ s^{-1}$. This fast dissociation rate is perfectly consistent with the requirements, which Schöneberg et al. (1) found to be necessary to fit the experimental data set (the authors estimated k_{-pre}, synonymously $k^{off}{}_{dark}$, to be necessarily at least 11,200 s^{-1}; see above).

However, Schöenberg et al. (1) employed as their k_{-pre} the absolute $k_{off} = 0.148\ s^{-1}$ measured in our SPR experiments, and pointed out that this far-too-low value would block the cascade, despite the fact that we emphasized in our article that only relative values of our SPR kinetics would give reasonable assumptions and results. We believe that the implementation of the relative values for the kinetic constants would be fully compatible with the experimental data used in Schöneberg et al. (1) for model tuning, as well as with a broad variety of other experimental data on whole-cell light response investigated for amphibian (5,8) and mouse rods (9) in our previous work. Also, this alternative implementation would further confirm and strengthen what we have already concluded in our previous study (5): nonproductive complexes between dark R and G should be intrinsically highly transient, and form and dissociate significantly quickly in order to be compatible with the phototransduction kinetics.

On the role of RG preformed complexes within a realistic supramolecular framework

We further believe that the simulations by Schöneberg et al. (1) cannot be conclusive as to the role of preformed RG complexes for another important reason. The authors limit the investigation of the role of RG complexes to the classical framework of R and G freely diffusing in disks and raise doubts about the existence of such precomplexes, which would simply slow down the rate-limiting steps in the cascade. It is also stated that under no circumstances can the existence of precomplexes increase the activation rate of G, independent of whether the activated R is initially precomplexed with a G. However, experimental evidence by atomic force microscopy and cryoelectron microscopy showed that R in native disk membranes is organized in supramolecular structures building heterogenous nanodomains or R rafts of different size (11,12,14)—an arrangement that has been very recently observed for murine and human rod outer segment disks (13) in addition to bovine disks.

It has been suggested that R is highly ordered within such nanodomains or rafts, and forms rows of dimers that interact with one another to form the raft (12–14). Schöneberg et al. (1) implemented the supramolecular organization of R into racks, which have been considered as noninteracting rows of R dimers of different length spread over the membrane surface, thus not accounting for the evidence that differently sized and shaped rafts may be formed by different assemblies of such units (see Fig. S8 in Schöneberg et al. (1)). It appears therefore not surprising that the diffusional mobility of both R and G was reduced in such a framework of immobile rows of dimers as it was predicted by simulations in Schöneberg et al. (1). Such a supramolecular arrangement would therefore need a compensatory mechanism to reproduce the classical diffusion constants that set the speed of the early steps in phototransduction (1). Schöneberg et al. (1) proposed that a faster lateral diffusion of G may compensate for immobile assemblies of R. However, would this argument withstand the implementation of the more realistic supramolecular scenario of rows of dimers forming rafts or nanodomains? Is there any other mechanism, alternative to lateral diffusion of G, that would guarantee the correct rate for the encounters between R* and G in such a framework?

We suggest that an alternative mechanism may exist. The transient and very fast dissociating RG complexes might indeed play a significant role in conditions in which assemblies of R cannot rapidly diffuse in the lipid milieu, and yet

need to rapidly find the cognate G protein to activate. This is substantially supported by our previous work proving that the nature of RG interactions in the dark is that of a pure protein-protein interaction (5), thus ruling out the involvement of lipids in mediating the interaction. We defined this putative mechanism as a dynamic scaffolding of G onto R: the protein-protein scaffolding is highly dynamic, as a combined result of the diffusion of G in the lipid milieu by its farnesyl and acyl modifications and the high rate of dissociation/association from/to dark R, when diffusion in the lipid membrane is prevented (5). We point out that the dynamic scaffolding mechanism based on very transient complexes between R and G per se does not make any assumption as to the intrinsic order of the R assemblies, although the kinetic advantage of structurally ordered organization of R within nanodomains or rafts has been anticipated by Monte Carlo simulations (2).

If this were the case, G might reach R* within such rafts without necessarily diffusing between the R molecules forming the rafts, but instead by "hopping" onto inactive R molecules in a very fast and transient fashion, according to the suggested dynamic scaffolding mechanism.

Is the evidence of transient complexes formed between dark R and G circumstantial and of little physiological relevance? The extremely high sensitivity of R to even single photons is setting a frame for specific cellular requirements, for which a scenario involving preformed RG complexes would be supportive as pointed out in a recent contribution (10). Finally, the successful integration of the data obtained by our SPR study into the up-to-date, most comprehensive kinetic model of phototransduction led us to conclude that RG transient complexes in the dark are fully consistent with the known kinetics of phototransduction and therefore very likely play a physiological role. Such preformed complexes between a GPCR and a G protein might be of importance to other GPCR systems as well by increasing efficiency or decreasing inherent noise.

Modeling the dynamics of a complex signaling pathway accounting for spatiotemporal information remains a tremendously difficult task. The main challenge is to build physically realistic microscopic models that allow the assessment of kinetic properties, which can then be analyzed at a system-level and compared with experimental data. The work by Schöneberg et al. (1) significantly improves previous attempts for the case of the diffusion and reactions occurring in photoreceptor disks, but for the reasons explained above, in our opinion it does not allow us to draw realistic conclusions as to the role of transient RG complexes in the physiology of phototransduction.

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Daniele Dell'Orco,[1,*] and Karl- Wilhelm Koch[2,*]

[1]Department of Life Sciences and Reproduction, Section of Biological Chemistry and Centre for BioMedical Computing, University of Verona, Verona, Italy; and [2]Department of Neurosciences, Biochemistry Group, University of Oldenburg, Oldenburg, Germany

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